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Salt-dependent surface activity and micellization behaviour of zwitterionic amphiphilic diblock copolymers having carboxybetaine

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ABSTRACT.

Zwitterionic amphiphilic diblock copolymers having *n*-butylacrylate(*n*-BA) and carboxybetaine as hydrophobic and hydrophilic blocks, respectively, with various chain lengths were synthesized by the reversible addition-fragmentation chain transfer (RAFT) polymerization method. The effects of salt and chain length on the solution behaviour of zwitterionic amphiphilic diblock copolymers were investigated using surface tension, static light scattering (SLS), dynamic light scattering (DLS) and fluorescence spectroscopic measurements. Surface tension measurements revealed that zwitterionic amphiphilic diblock copolymers undergo transition from surface active to non-surface active after addition of salt. This may be due to the transition from zwitterionic (almost zero charge) to ionic nature of the betaine block by addition of small amount of salt followed by an increase of solvent power of salt solution. Foam formation behaviour decreased with increasing chain length. The critical micelle concentration was decreased drastically by the presence of a small amount of salt, which reflected the non-surface active nature of block copolymer. Increase in values of hydrodynamic radii with increase in salt is attributed to stretching of the hydrophilic chain due to the ionic nature of the block chain and the increase of solvent power. Aggregation number (N_{agg}) and the second virial coefficient (A_2) decreased with the increase in chain length as well as after addition of salt. Packing parameters of block copolymer micelles were less than 0.3 which implies that the micellar shape is spherical. Some unique aspectes of zwitterionic polymer were elucidated, which are largely different from simple ionic (anionic and cationic) polymers.

INTRODUCTION

Surface activity and micellization behaviours of non-ionic and ionic amphiphilic diblock copolymers have been extensively studied theoretically [1-4] and experimentally [5-21]. Adsorption behaviours of block copolymers at interfaces in selective and non-selective solvent have been investigated [1, 2]. Joanny *et al.* [3] reported that adsorption of ionic amphiphilic diblock copolymers at the air-water interface occurs if the fraction of charged monomers in the polyelectrolyte block is smaller than the critical value. However, if the charge fraction is larger than the critical value, the osmotic pressure in the adsorbed layer is too large and the copolymers do not adsorb (or only weakly adsorbs) at the air-water interface.

We have extensively investigated the non-surface activity and micellization behaviour of anionic and cationic amphiphilic diblock copolymers in aqueous media [5-15]. These studies showed that both anionic and cationic amphiphilic diblock copolymers behave like non-surface active polymers though they form micelles in aqueous solutions. Non-surface active behaviour, i.e, almost no adsorption of ionic amphiphilic diblock copolymers at the air-water interface have been explored by X-ray reflectivity studies [5-9]. It has been clarified that the non-surface active nature of ionic amphiphilic diblock copolymers at the air-water interface depends on some conditions such as block length, block ratio, total length, and ionic strength. For more details, refer to our previous papers [8, 14, 15]. Non-surface active nature of ionic amphiphilic block copolymers has also been reported by several researchers. Tirrell and his co-workers [16] monitored the adsorption of P(*t*-BS)-*b*-NaPSS on silica surfaces using *in-situ* ellipsometry studies and it was found that there was no adsorption without salt whereas salt addition leads to adsorption. Thomas and Armes *et al*. [17, 18] used neutron reflectivity to study the effects of composition, molecular weight, pH and ionic strength on the adsorption of ionic amphiphilic diblock copolymers at the air-water interface. Hoffmann and Müller *et al.*[19] studied the surface activity of the polymer (PBA-*b*-PAA) solution with

increasing degree of neutralization and observed that an abrupt transition in surface activity from surface active to non-surface active at 10% neutralization. Laschewsky *et al*. [20] examined the surfactant properties of amphiphilic diblock copolymers with the same hydrophobic block and various ionic and non-ionic hydrophilic blocks. We have proposed that the main reason for this non-surface activity is the image charge effect at the air-water interface [21] and very high stabiliy of polymer micelles in bulk solution [7, 8, 11-15]. Since the anionic and cationic amphiphilic diblock copolymers contain polyions, the electrostatic repulsion between these polyions and the water surface by an image charge effect is very strong at the air-water interface. This effect destabilizes the adsorption of polymers at the air/water interface and also lowers the critical micelle concentration (CMC) more which is originally very low because of the polymeric nature of the micelles. As a result, ionic amphiphilic diblock copolymers are not adsorbed at the air/water interface; polymer micelle is formed before polymer adsorption occurs.

Zwitterionic polymers are attracting attention because of their unique characters by zwitterionic nature and also their high biocompatibility. However, studies on the surface activity and micellization behaviour of zwitterionic amphiphilic block copolymers are limited [22-25]. In addition, characterization of amphiphilic block copolymers having carboxybetaine has been underexplored. In this context, we studied zwitterionic amphiphilic block copoymers, using carboxybetaine as a zwitterionic block. Since betaine contains both anionic and cationic groups, its property is expected to be largely different from conventional ionic systems, i.e., anionic and cationic amphiphilic block copolymers. Hence, it is interesting to study the fundamental molecular properties and the self-assembly behaviours of amphiphilic diblock copolymers containing carboxybetaine. Here, we examined the effect of salt and chain length on its surface activity and micellization behaviour by the surface tension, static light scattering (SLS), dynamic light scattering (DLS) and fluorescence probe measurements.

EXPERIMENTAL SECTION

Materials. *n*-Butylacrylate (*n*-BA), 4,4′-Azocyanovaleric acid (ACVA), 2,2′-azobisisobutyronitrile (AIBN), *N,N*-dimethylformamide (DMF), diethyl ether, methanol and *N*-phenyl-1-naphthylamine (NPN) were purchased from Wako Pure Chemicals (Osaka, Japan). Carboxybetaine (GLBT) was a kind donation from Osaka Organic Chemicals Co. Ltd. (Osaka, Japan). *n*-BA was purified by distillation. 4-Cyanopentanoic acid-4-dithiobenzoate was used as chain transfer agent (CTA), which was synthesized as reported [26]. Deuterium oxide (D_2O), deuterated methanol (CD_3OD) and deuterated chloroform ($CDCl_3$) were products of Cambridge Isotope Laboratory (U.K.). Milli-Q System (Millipore, Bedford, MA) was used to obtain ultrapure water of resistance 18 MΩ cm, which was used for solution preparation and dialysis.

Synthesis. Reversible addition-fragmentation chain transfer polymerization (RAFT) was used to synthesize both homopolymers (PGLBT) and diblock copolymers (P(*n*-BA)-*b*-PGLBT) with various block lengths (Scheme 1). Table 1 lists the polymerization conditions used for the synthesis of homopolymers (PGLBT). The PGLBT homopolymer was synthesized by initially mixing GLBT, CTA and ACVA with the mixed solvents of water and DMF at a volume ratio of 4:1 in a Schlenk tube using a magnetic stirrer. Then the mixture was subjected to degassing with three cycles of freeze-pump-thaw and then the tube was filled with Ar gas. RAFT polymerization was carried out at 70° C for 2hrs. After polymerization, the product was dialyzed against Mill-Q water for 3 days and the solid PGLBT homopolymer was obtained by freeze-drying. The molecular weight and distribution of PGLBT were determined by GPC. The molecular properties of PGLBT thus obtained are summarized in Table 2. Successful synthesis of homopolymers (PGLBT) was confirmed by ^{1}H NMR measurements (Figure 1).

The polymerization conditions used for the synthesis of diblock copolymers (P(*n*-BA)-*b*-PGLBT) are listed in Table 1. For synthesis of diblock copolymers, (P(*n*-BA)-*b*-PGLBT), *n*-BA, PGLBT (also macro CTA for block copolymer) and AIBN were dissolved in methanol and mixed well in a Schlenk tube using a magnetic stirrer. After degassing the mixture with three cycles of freeze-pump-thaw, RAFT polymerization was carried out at 70 °C for 24 hrs. Diblock copolymers were separated from diethyl ether by precipitation. By ^{1}H NMR measurements (Figure 1), the block lengths of P(*n*-BA) were determined and listed in Table 2.

^{1}H Nuclear magnetic resonance (^{1}H NMR). ^{1}H NMR spectra were recorded using a JEOL 400WS (JEOL, Japan). For obtaining the ^{1}H NMR spectra of homopolymers (macro CTA-PGLBT), D_2O was used as a solvent. For diblock copolymers, i.e., P(*n*-BA)-*b*-PGLBT, mixed solvents of CD_3OD and $CDCl_3$ at a volume ratio of 1:1 were used. All measurements were carried out at room temperature.

Gel permeation chromatography (GPC). GPC measurements with aqueous buffer as an eluent were carried out using a JASCO system LC-2000 with a UV detector (UV-2075), a refractive index detector (RI-2031) and Shodex OH pack (SB-804 HQ). The eluent was a mixture of 0.3 M sodium sulfate (Na_2SO_4) and 0.5 M acetic acid (CH_3COOH) whose pH was around 3. The concentration of the sample solution injected was ca. 2 mg/mL. The number averaged molecular weight (M_n) and the polydispersity index (M_w/M_n) of PGLBT (macro CTA) were determined.

Surface tension measurements. Surface tension measurements were conducted at room temperature and by Wilhelmy plate (platinum) method with a FACE CBVP-Z Surface Tensitometer from Kyowa Interface Science Co., Ltd. (Tokyo, Japan).

Foam formation and foam height measurements. For foam formation observation, 1 mg/ml of block copolymers aquous solutions were prepared using Milli-Q water with and without salt (1 M NaCl). Then these polymer solutions were mechanically shaken for 1 min in identical containers. The polymer solutions were photographed and also the height of the foam was measured as a function of time.

Light scattering measurements. SLS and DLS measurements were carried out at 25 °C using a Photal SLS-7000 light scattering goniometer system (Otsuka Electronic, Osaka, Japan) equipped with a multi-τ correlator (GC-1000) and a 15 mW He-Ne laser (wavelength: 632.8 nm). The time averaged intensity-intensity autocorrelation function (ICF) $g^{(2)}(q,t)$ was measured at different scattering wave vectors $q = (4\pi n/\lambda)\sin(\theta/2)$; where n is the refractive index of the solvent, θ is the scattering angle and λ is the wavelength of incident laser beam. The field correlation function $g^{(1)}(q,t)$ was obtained from ICF $g^{(2)}(q,t)$ using Siegert relation [27]. The decay rate Γ was extracted from the field correlation function $g^{(1)}(q,t)$ by single or double-exponential fitting. From the plot of Γ vs. q^2, the translational diffusion coefficient (D) was evaluated using the relation, $\Gamma = Dq^2$). Then, from the obtained D value, the hydrodynamic radius (R_h) was calculated using the Stokes-Einstein equation.

SLS measurements were performed by varying the scattering angle (θ) from 30° to 150° at 10° intervals. The weight-average molecular weight (M_w), radius of gyration (R_g) and the second virial coefficient (A_2) were extracted from Zimm plots using the following equation [28, 29]

$$\frac{KC_p}{I(q)} = \frac{1}{M_w}\left(1 + \frac{1}{3}\langle R_g \rangle^2 q^2\right) + 2A_2C_p + \ldots \qquad (1)$$

Where, K is an optical constant ($4\pi^2 n^2 \left(dn/dC_p\right)^2 / N_A \lambda^4$), $I(q)$ is the scattered intensity at given q, C_p is polymer concentration, dn/dC_p is the refractive index increment against C_p and N_A is Avogadro's number.

Specific refractive index increment measurements (dn/dc_p). The specific refractive index increment (dn/dc_p) was determined using the Photal differential refractometer (Otsuka Electronic, Osaka, Japan) operating at a wavelength of 632.8 nm. These measurements were done at 25 °C.

Fluorescence measurements. Fluorescence spectra were recorded with the HITACHI F-2500 fluorescence spectrometer (Tokyo, Japan) in the right-angle geometry (90° collecting optics) with a bandwidth of 2.5 nm. For NPN, the samples were excited at 340 nm and emission spectrum was recorded between 350 and 550 nm. Since the fluorescence probe (NPN) is insoluble in water, it was dissolved in methanol to obtain 1 mM stock solution. Then the stock solution was diluted to 1 μM by adding various concentrations of polymer solution. The final solution was allowed to reach equilibrium for 1 day prior to fluorescence measurements. Fluorescence measurements were performed at 25 °C.

RESULTS AND DISCUSSION

Surface active to non-surface active transition. As mentioned earlier, so far we have focussed on the investigation of non-surface activity and micellization behaviour of ionic amphiphilic diblock copolymers. Our previous studies on anionic and cationic amphiphilic diblock copolymers showed that ionic amphiphilic diblock copolymers are non-surface active when some requirements are satisfied. They are not adsorbed at the air-water interface, but they can self-assemble into micelles in aqueous media [5-15]. The primary origin is a synergetic effect of an image charge effect and very high stability of the polymer micelle in bulk solution: the image charge effect destabilizes polymer adsorption at the air/water

interface which results in lowering the CMC. Thus, micellization occurs before adsorption occurs. In the present work, we investigated the molecular properties of amphiphilic diblock copolymers having zwitterion block which has both anionic and cationic charges together. As the first target, we have chosen carboxybetaine as zwitterionic block and examined the effect of salt and chain length on the surface active and micellization behaviour of zwitterionic amphiphilic diblock copolymers using surface tension and light scattering techniques.

Figure 2 shows the variation of surface tension of zwitterionic amphiphilic diblock copolymers with various hydrophobic and hydrophilic chain lengths as a function of their concentrations in water and in aqueous sodium chloride solutions with different concentrations. It is clear from this figure that in the absence of salt, the surface tension of the solution decreased with increasing polymer concentration, although it did not vary drastically, for all block copolymers studied here. This means that zwitterionic block copolymers are adsorped at the air-water interface in higher concentration regions; zwitterionic amphiphilic block copolymers behave like weakly surface active polymers in water. Earlier studies showed that the surface activity of block copolymers having sulfobetaine was weak [23]. Previous studies on anionic and cationic amphiphilic block copolymers [5-15] revealed that the non-surface activity is suppressed by salt addition. This means that the electrostatic image charge effect plays an important role in non-surface activity. However, there is less decrease in magnitude of surface tension of zwitterionic amphiphilic block copolymers with increase in polymer concentration when salt is added. This is opposite that observed with anionic and cationic non-surface active polymers, and implies that the addition of small amount of salt causes the zwitterionic amphiphilic block copolymers to undergo transition from weakly surface active to non-surface active. It is assumed that the zwitteriionic block behaves as an almost neutral polymer since cation and anion in the molecule forms an inner

salt in pure water. Hence, the electrostatic effect is not dominant, so it is quite reasonable that the block copolymer behaves as a neutral amphiphilic block copolymer in water, which is surface active. However, the observation after salt addition here certainly means that the zwitterionic block copolymer undergoes transition from surface active to non-surface active. This suggests that the zwitterionic block, which is almost neutral in pure water, changes to ionic by salt addition. The cation and anion in the zwitterionic block, which originally form the inner salt, become two salt ions after addition of salt with each salt ion as a counterion. At this moment, the ionic nature of zwitterions in salt solutions has not been confirmed, but it might be reasonable to think that the zwitteionic block is weakly cationic in salt solutions since the quaternary ammomium cation is a strong base while carboxylic acid is a weak acid. However, our recent observations showed that the quaternary ammonium cation behaves like a weak base and hence betaine may show anionic nature [12, 13]. Tottaly anionic nature of carboxybetaine was reported by several researchers [30-33] and also by Saurabh et al. recently. It should be noted that the polymer shows non-surface active nature even at higher added salt concentration such as 0.5 M and 1M NaCl. Since the image charge repulsion, an electrostatic effect at the air/water interface, is one of the important factor for non-surface active nature, anionic and cationic non-surface active polymers transfered to surface avtive by large amount of salt. [7,8,10,11] Hence, apparently non-surface active nature of zwitterionic block copolymers are unique character and its origin should be different; an increase of solvent power of salt solutions for zwitterionic block might be a key factor of this phenomena.

We also examined the influence of salt and block length on foam forming behaviours of zwitterionic amphiphilic block copolymers in solution. The foam-forming behaviour of zwitterionic amphiphilic block copolymers with various chain lengths in the absence and presence of 1M sodidum chloride solutions is shown in Figure 3. In the absense of added salt,

foam formation ability seems to decrease with increase in chain length of block copolymer. This means that higher molecular weight block copolymers are more non-surface active than those with a lower molecular weight. This is in good agreement with our finding that there is a lower limit of molecular weight for non-surface activity [14, 15]; 20:20 polymer was surface active while the 50:50 polymer was non-surface active. Also, the surface tension data in Fig.2 shows less decrease of surface tension for *n*-BA_{254}-*b*-$GLBT_{300}$ polymer than other polymers. These observations certainly support our opinon that the non-surface activity is due to a kind of polymer effect.

However, addition of salt slightly affects the foam formation for long chain polymers while almost no influence was observed for short chain polymers. Although the transition from weakly surface active to non-surface active is obvious in Fig.2, the increase of surface tension by salt addition is not so large. Hence, not so drastic change of foam formation behaviour by salt addition is not unnatural. More foam, although not so much, after addition of salt for *n*-BA_{254}-*b*-$GLBT_{300}$ polymer might be related to the incerase of solvent power of 1M NaCl aq. for betaine block.

Some solutions appear bluish especially *n*-BA_{47}-*b*-$GLBT_{55}$ and *n*-BA_{254}-*b*-$GLBT_{300}$ polymers. This means that micelle formation whose size is in the range of wavelength of visible light. In other words, some aggregates are formed in solution but they are not so large, imhomogeneous aggregates. For the size and properties of micelles will be examined later. Figure 4 shows the variation of foam height of the block copolymer as a function of time. Foam height of the block copolymer with a shorter chain length is higher than that of a block copolymer with a longer chain length. These observations are in good agreement with our observations shown in Figures 2 and 3; i.e., block copolymers with a longer chain length are more non-surface active than block copolymers with a shorter chain length.

Micellization behaviour of block copolymers in the absence and presence of salt

CMC as a function of salt concentration. In order to confirm whether these zwitterionic amphiphilic block copolymers form micelles or not, we investigated the micellization behaviours of these copolymers using SLS and fluorescence probe measurements. This is because in some cases where the block copolymers having a very low critical micelle concentration (CMC), determining the CMC by SLS becomes difficult and sometimes its reliability becomes less due to the very low count rate. On the other hand, the fluorescent probe method has higher sensitivity at a low polymer concentration for CMC determination while there is some uncertainty that the fluorescence probe added might change CMC itself. Hence we employed both techniques in this study. CMC of zwitterionic amphiphilic block copolymer (*n*-BA_{104}-*b*-$GLBT_{156}$) as a function of sodium chloride concentration by SLS is shown in Figure 5. Table 3 lists the values of CMC of different block copolymers obtained from SLS and fluorescence probe measurements. Addition of 0.1 M NaCl decreased CMC drastically and further addition of salt had little effect. It is well known that CMC of normal, low molecular weight ionic surfactants decreases with increasing added salt concentration [34]. This is famous the Corrin-Harkins law. Our observation here for zwitterionic block copolymer may seem to be discrepant with this famous law. However, this is in good agreement with our previous observations for non-surface active polymers. The CMC of non-surface active polymers increases with increasing salt concentration. We have shown that zwitterionic amphiphilic block copolymers are weakly surface active in water but almost non-surface active in salt solutions. An apparence of non-surface active nature by addition of small amount of salt can be interpereted by an increse of ionic nature of zwitterionic betaine block. Non-surface active nature at higher salt concentration conditions, where an image charge effect should not play an important role, might be due to an increase of stability of

polymer micelle in bulk solution; stronger solvent power of salt solutions for betaine block should increase the micelle stability, which has zweitterionic betaine block chains as a corona. Hence, our observation here, i.e., lower CMC in salt solution than in pure water is in good agreement with transition from surface active to non-surface active by salt addition.

Figure 6 shows the variation of fluorescence intensity of NPN probe as a function of polymer concentrations with and without salt for different zwitterionic amphiphilic diblock copolymers. The fluorescence intensity is very weak at a low polymer concentration, but with increasing polymer concentration, the fluorescence intensity increased markedly up to a certain polymer concentration. This bending point corresponds to CMC since this means that the NPN fluorescence probe is resolubilized in a hydrophobic core, i.e., micelle formation occurs while almost no NPN is resolubilied at a low polymer concentration because there is no micelle in solution (only unimers). This figure also shows that CMC of zwitterionic amphiphilic diblock copolymers decreases by addition of salt, which is consistent with SLS results. Also it is observed that CMC depends on the net hydrophobicity of the block copolymers although the difference of CMC is not so large. The higher the hydrophobicity the lower the value of CMC of block copolymer; CMC of *n*-BA_{88}-*b*-$GLBT_{117}$ looks highest. In addition, fluorescence intensity also depends on the net hydrophobicity of block copolymers; i.e., the fluorescence intensity of *n*-BA_{88}-*b*-$GLBT_{117}$ is lower than those of (*n*-BA_{47}-*b*-$GLBT_{55}$ and *n*-BA_{254}-*b*-$GLBT_{300}$.

Salt concentration dependence of hydrodynamic radius of micelle. Since the formation of micelles has been confirmed by SLS and florescence probe measurements, DLS has been used to determine the hydrodynamic radius of the micelles that are formed by zwitterionic amphiphilic block copolymers. Effect of polymer and salt concentrations on hydrodynamic

radius has been investigated. Variation of hydrodynamic radius (R_h) of the block copolymer micelle as a function of polymer concentrations in the absence and presence of salt at various concentrations is shown in Figure 7. Hydrodynamic radius of the block copolymer micelle was independent of polymer concentration as was observed previously. On the other hand, what is interesting is that the salt concentration dependence of R_h shown in inset of Figure 7. In the case of ionic (anionic or cationic) amphiphilic block copolymers, the R_h was not affected largely after addition of a high concentration of salt [5, 7-9] up to a certain salt concentration. However, the increase of salt concentration caused an increase of R_h in the zwitterionic block. The addition of salt causes the zwitterionic block to change from almost neutral to slightly ionic. Hence, this can be interpreted by the stretch of zwitterionic betaine block due to the increase of electrostatic repulsion. However, at a higher salt concentration, the electrostatic repulsion to stretch the corona chain is not so strong due to the electrostatic shielding effect. Hence, it is reasonable to think that the corona chains expanded with an increase of solvent power of high salt solutions. Armes *et. al.* [23] observed similar behaviour for block copolymers containing sulfobetaine after addition of salt. In their study, the hydrodynamic radius of the micelle increased with increasing salt concentration up to 0.1M NaCl and then slightly decreased with further increase of salt concentration.

The dissymmetry ratio depends on the symmetry between hydrophobic and hydrophilic block lengths. The higher the symmetry between hydrophobic and hydrophilic block lengths the lower the dissymmetry ratio. Also the dissymmetry ratio increased with the increase in chain length of block copolymer (Table 4). Addition of salt slightly affects the dissymmetry ratio. Polydispersity of the micelles in the present study depends on the symmetry between hydrophobic and hydrophilic blocks. Higher the symmetry of block copolymer lowers the polydispersity of hydrodynamic radius. For instance, the polydispersity of the micelles that formed from the block copolymers *n*-BA_{47}-*b*-$GLBT_{55}$ and *n*-BA_{254}-*b*-

$GLBT_{300}$ are less in comparison with the other two block copolymers *n*-BA_{88}-*b*-$GLBT_{117}$ and *n*-BA_{104}-*b*-$GLBT_{156}$ (see Table 4). These results are consistent with the bluishness of the solution.

Effect of salt and block length on the structure of micelles. In order to describe the structure of micelles, parameters related to their structures such as weight-average molecular weight (M_w), aggregation number (N_{agg}), the second virial coefficient (A_2) and radius of gyration (R_g) have been extracted from Zimm plots as shown in Tables 5 and 6. Figure 8 shows typical Zimm plots for the block copolymers in aqueous 1M NaCl solutions. The aggregation number (N_{agg}) in water decreased from 755 to 155 with the increase in block length of P(*n*-BA) from 47 to 254 and block length of PGLBT from 55 to 300. This is similar to previous findings obtained with different micellar systems such as PS-*b*-P4VPEtBr [35], PS-*b*-PEO [36] and PS-*b*-PANa [37]. In those cases, the aggregation number decreased with the increase in length of the soluble block. Furthermore in the present work, salt addition decreased the aggregation number to 69 and 35, respectively, for *n*-BA_{47}-*b*-$GLBT_{55}$ and *n*-BA_{254}-*b*-$GLBT_{300}$. Armes *et. al.* [23] also observed analogous phenomenon for their block copolymers having sulfobetaine. The second virial coefficient (A_2) of *n*-BA_{47}-*b*-$GLBT_{55}$ and *n*-BA_{254}-*b*-$GLBT_{300}$ was 5.4 $x10^{-5}$ and -4 $x10^{-7}$ $cm^3g^{-2}mol$, respectively, in water. The decrease in value of A_2 with increase in chain length could be due to the decrease in polymer-solvent interaction or decrease in polymer solubility. In addition, after salt addition the value of A_2 of *n*-BA_{47}-*b*-$GLBT_{55}$ and *n*-BA_{254}-*b*-$GLBT_{300}$ are decreased to 0.9 $x10^{-5}$ and -2.4 $x10^{-5}$ $cm^3g^{-2}mol$, respectively. This decrease in value of A_2 with increase in salt concentration is attributed to the interaction between the polymer and solvent. This behaviour could be responsible for decrease in CMC with increase in salt concentration.The decrease in CMC is due to the non-surface-active nature of the polymer in salt solutions. In general, CMC of a non-surface-active polymer is very low. Betaine corona stretched by salt addition. This is due

to increase of ionic nature of betaine block. Betaine block becomes ionic by salt addition, so the polymer became non-surface active. If the ionic nature of betaine corona block inceased, it might be reasonalbe to think A_2 increase because of enhancement of electrostatic repulsion between micelles. However, it is reasonable to think that further extention of betaine block with higher salt is due to increase of solvent power of salt soluion Hence, the decrease of A_2 is not unreasonble observation.

Radius of gyration (Rg) increased with increase in chain length and addition of salt had little effect (Table 6). This behaviour is anticipated because R_g is a measure of end-to-end distance between polymer chain and hence increase in R_g was observed with increase in chain length of block copolymer. Eisenberg and his co-workers [37] reported a similar trend of increasing R_g for their polymers.

By knowing the values of M_w and N_{agg}, the core radius (R_c) of micelles can be determined from the following equation [37]

$$R_c = \sqrt[3]{\frac{3N_{agg}N_{BA}M_{w(BA))}}{4\pi\rho N_A}} \quad (2)$$

where N_{agg}, N_{BA}, $M_{W(BA)}$, ρ, and N_A are the polymer aggregation number of block copolymers for each micelle, the degree of polymerization of *n*-BA, the molecular weight of *n*-BA, the density of bulk P(*n*-BA) (approximately 1.08 mg/mL) and the Avogadro constant, respectively. Packing parameter (β) of final aggregate structure can be calculated from the following equation [38, 39]

$$\beta = \frac{V_H}{L_C A_0} \quad (3)$$

where V_H, L_C and A_0 are volume occupied by hydrophobic chain, hydrophobic chain counter length(≈core radius, R_C) and the surface area per hydrophilic chain, respectively. The β

values predicts the morphology of polymer micelles; spherical micelle ($0<\beta<1/3$), rod-like micelle ($1/3<\beta<1/2$), vesicle ($1/2<\beta<1$), and planar bilayer ($\beta>1$). Packing parameters have been estimated from Eq. (3) using the values of V_H ($=4\pi R_c^3/3N_{agg}$), L_c ($=R_c$) and A_0 ($=4\pi R_h^2/N_{agg}$) for P(*n*-BA)-*b*-PGLBT in water and aqueous 1M NaCl solution. The β values thus estimated were less than 0.3 (Table 6), indicating spherical micelle formation.

Figure 9 shows the schematic representation of an effect of salt on the surface activity and micellization behaviours of zwitterionic amphiphilic diblock copolymers studied here. Salt addition causes the zwitterionic amphiphilic diblock copolymers to undergo transition from surface active to non-surface active. At the same time, the hydrodynamic radius of the micelle increased with increasing salt concentration while the aggregation numbers of the micelles decreased. All these observations can be reasonably explained by assuming that the addition of salt increases the ionic nature of the zwitterionic block , and further increase of solvent power by further addition of salt.

CONCLUSIONS

Zwitterionic amphiphilic diblock copolymers having various block lengths of poly(*n*-buthylacrylate) and poly(carboxybetaine) blocks were synthesized by the RAFT method. Influence of salt on the molecular nature and micellization behaviour of these block copolymers were studied by surface tension, SLS, DLS and fluorescence probe experiments. Zwitterionic amphiphilic diblock copolymers undergo transition from surface active to non-surface active by salt addition, which are reminiscent of an increase of ionic nature of hydrophilic betaine block by salt addition. This is in contrast to our previous finding that addition of salt caused transition of ionic (both anionic and cationic) amphiphilic diblock copolymers from non-surface active to surface active [5-15]. The CMC of these diblock copolymers decreased with increasing salt concentration, which is consistent with the non-

surface active nature of the polymers in salt solutions. DLS studies showed that the hydrodynamic radius increased with increasing salt concentration. This could be due to the increase in the chain length of hydrophilic corona because of the ionic nature of the betaine block in salt solutions at a low salt condition and an increase of solvent power with further increase of salt concentration. This is also in good agreement with the decrease in aggregation number of the micelle and also with the decrease in the second virial coefficient after addition of salt. Smaller values of packing parameter ($\beta < 0.3$) imply the formation of spherical nature of the micelles. This study showed that the surface activity and micellization behaviours of zwitterionic amphiphilic diblock copolymers are quite different from those of anionic and cationic amphiphilic diblock copolymers in the presence and absence of salt. The present findings should help developments and novel applications of betaine containing block copolymers.

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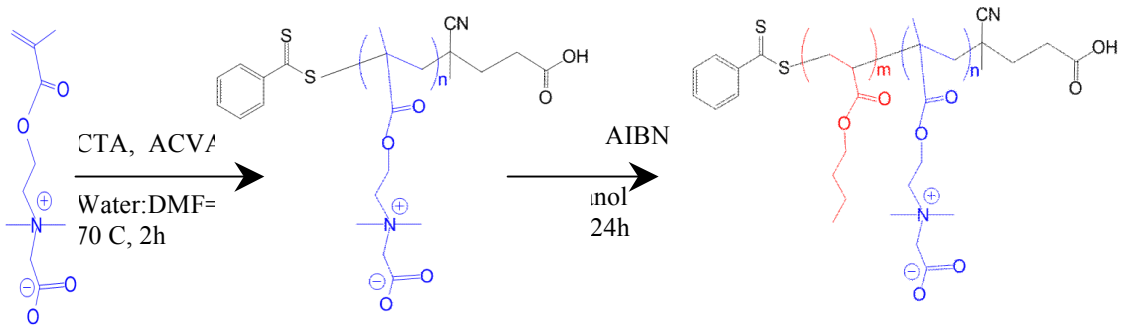

Scheme 1. Scheme of synthesis of homopolymer(PGLBT) and diblock copolymer(P(*n*-BA)-*b*- PGLBT)

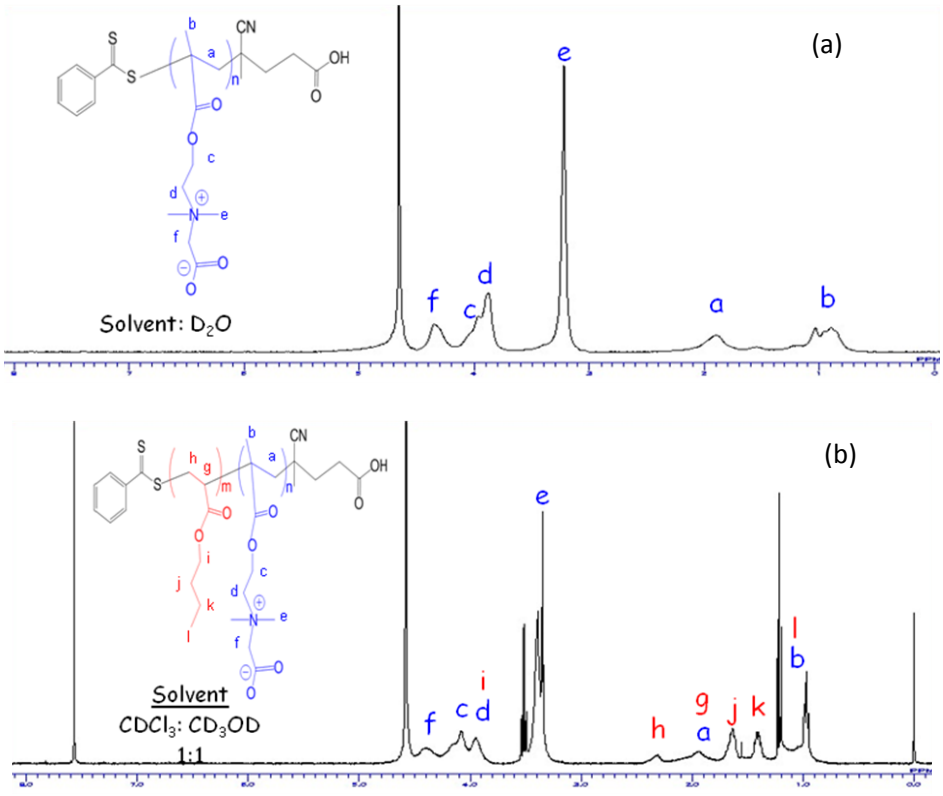

Figure 1

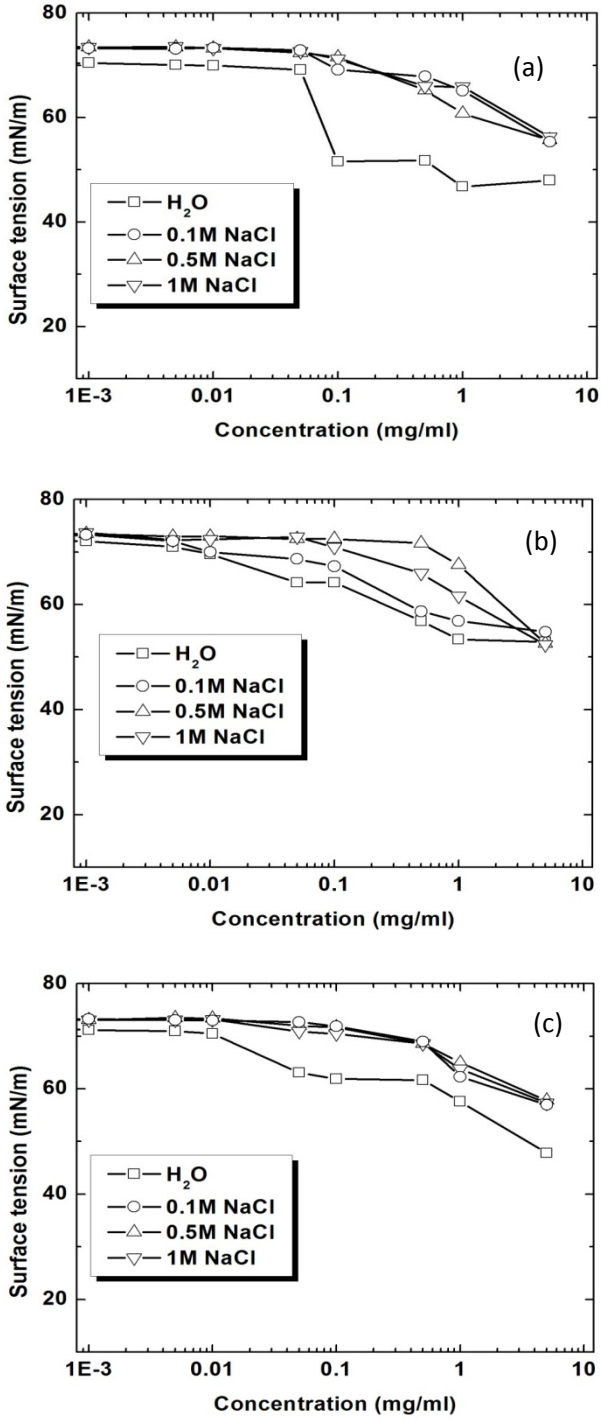
(a)
(b)
(c)
Surface tension (mN/m)
Concentration (mg/ml)
80
60
40
20
1E-3
0.01
0.1
1
10
H2O
0.1M NaCl
0.5M NaCl
1M NaCl

Figure 2

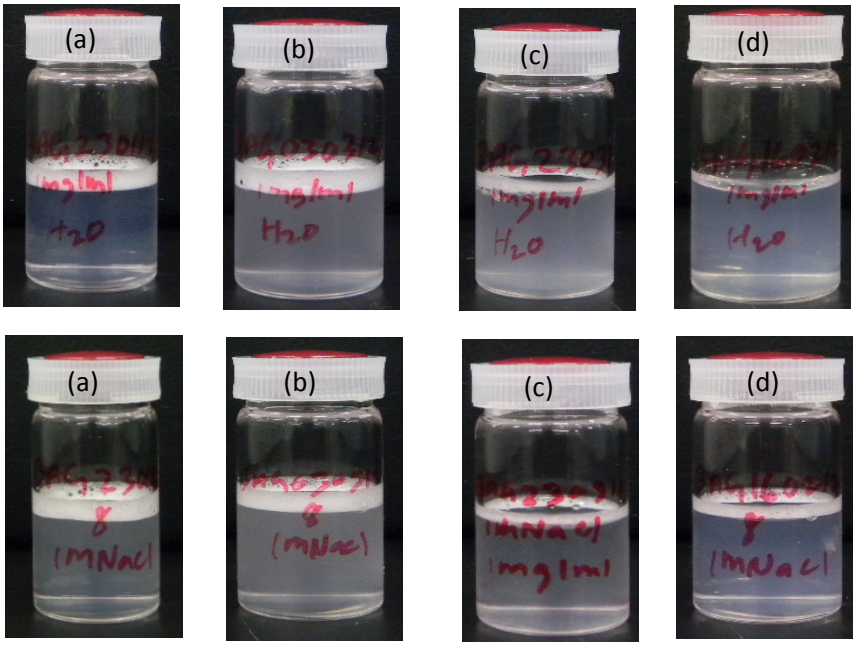

Figure 3

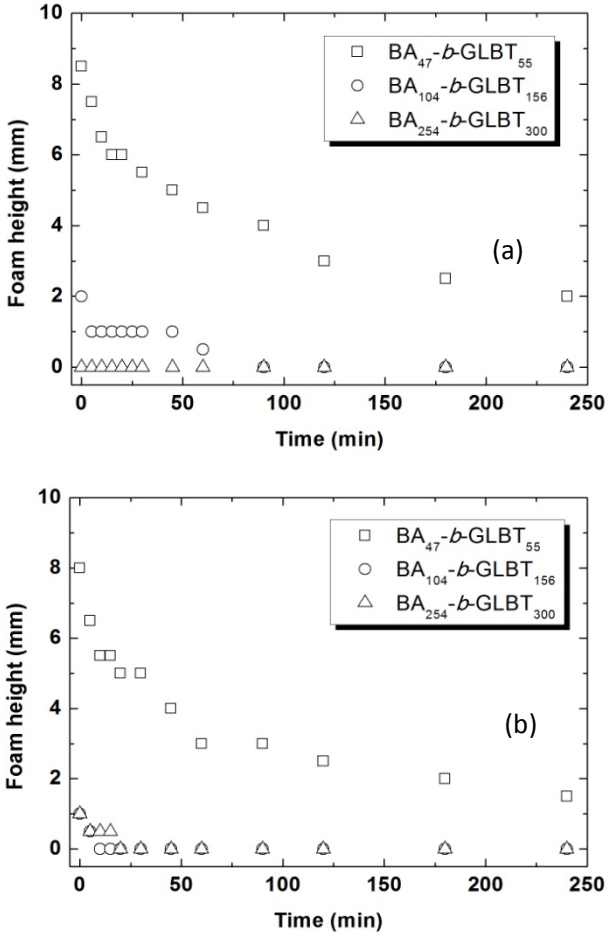

Figure 4

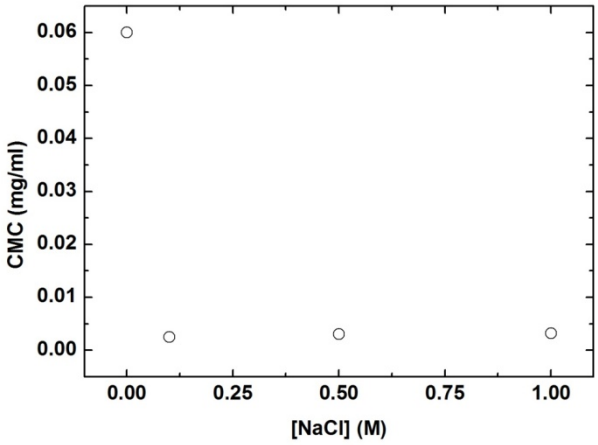

Figure 5

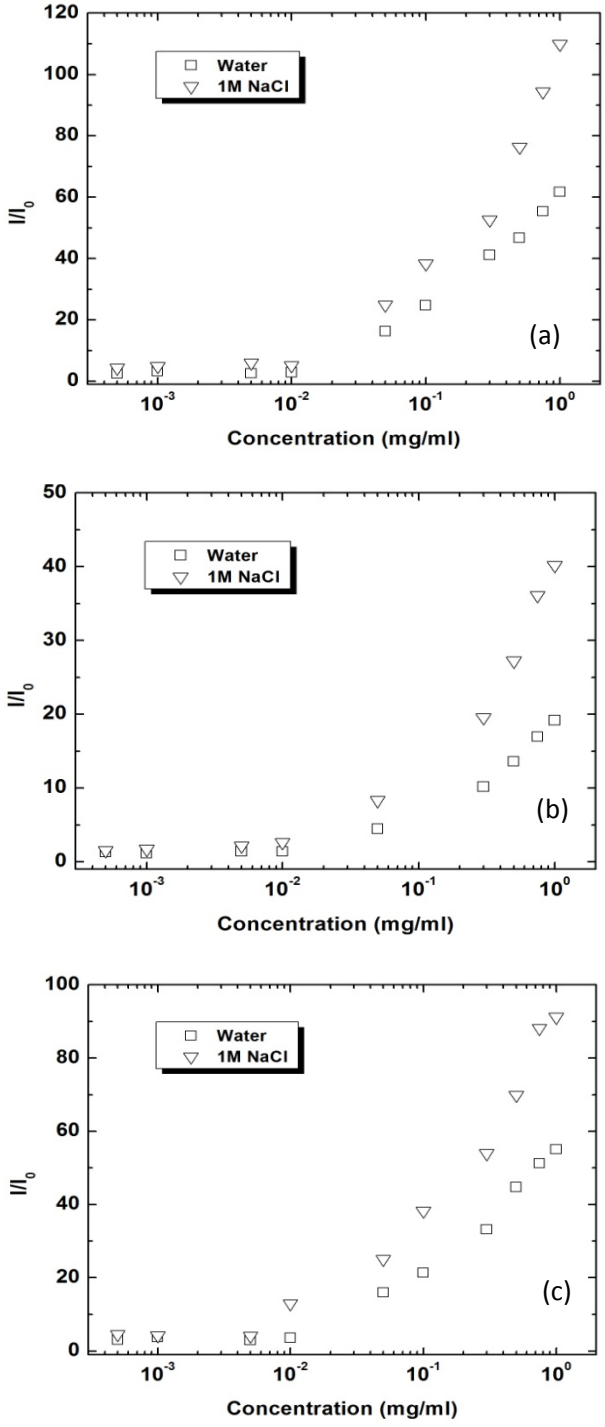

Figure 6

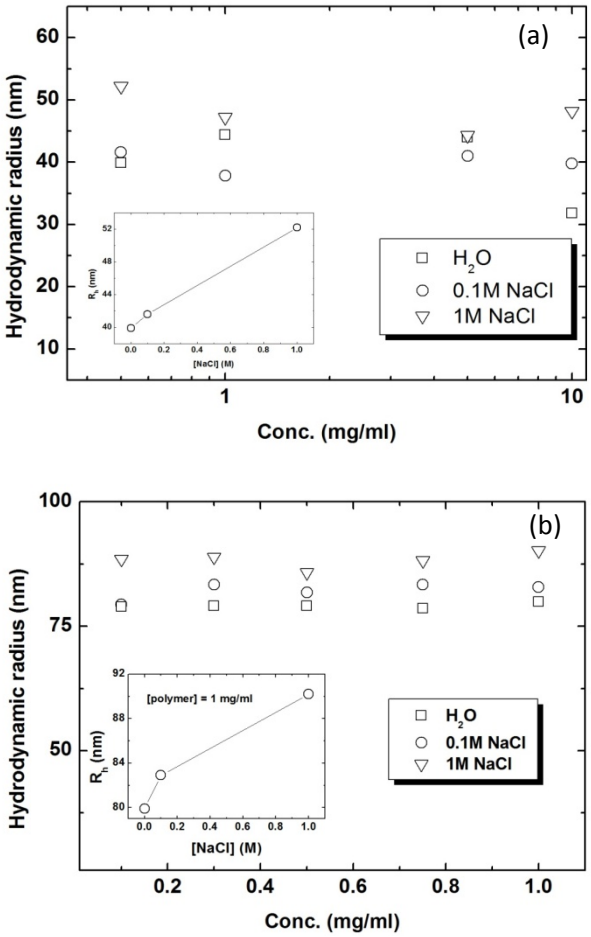
(a)
Hydrodynamic radius (nm)
Conc. (mg/ml)
H2O
0.1M NaCl
1M NaCl
Rh (nm)
[NaCl] (M)
(b)
Hydrodynamic radius (nm)
Conc. (mg/ml)
[polymer] = 1 mg/ml
Rh (nm)
[NaCl] (M)
H2O
0.1M NaCl
1M NaCl

Figure 7

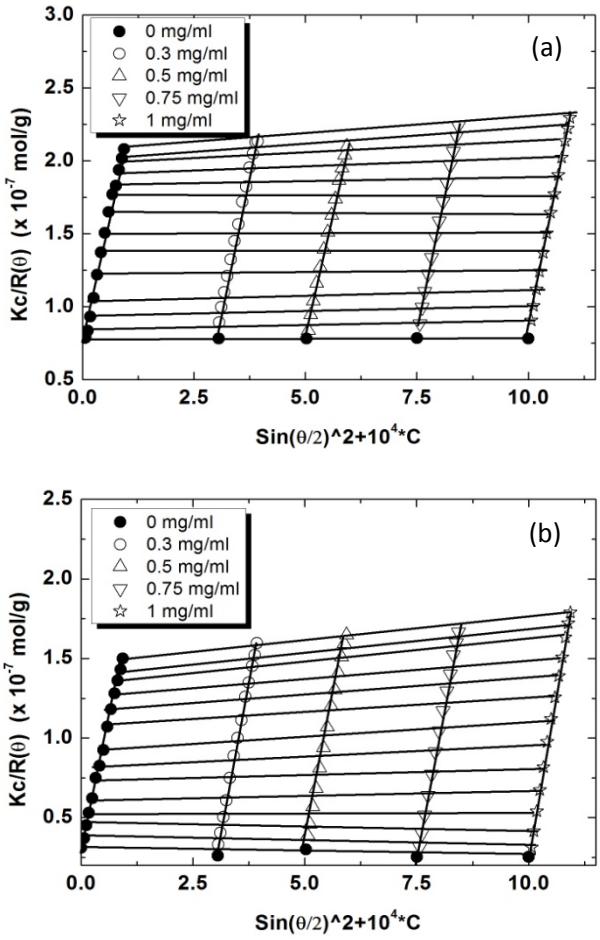

Figure 8

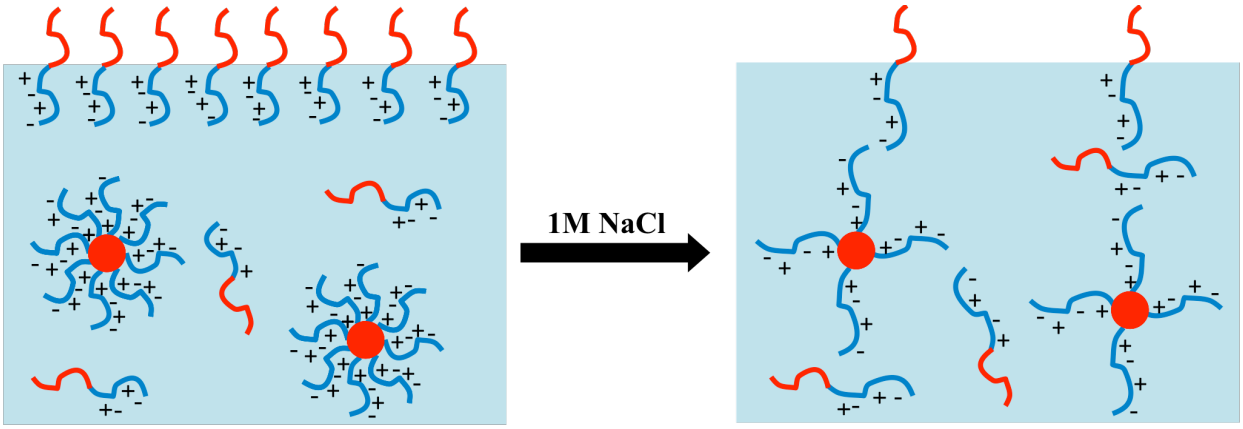

Figure 9

Table 1. Polymerization conditions for the synthesis of homopolymers (PGLBT) and block copolymers (P*n*-BA-*b*-PGLBT) (70°C)

Polymer	Monomer (mmol)	RAFT agent (mmol)[a]	Initiator (mmol)[b]	Water (ml)	DMF (ml)	Methanol (ml)	Polymerization time (hrs)
PGLBT – 1	18.6	0.2	0.1	6	1.5		2
PGLBT – 2	37.2	0.2	0.1	6	1.5		1.5
PGLBT – 3	37.2	0.19	9.7×10^{-2}	6	1.5		2
PGLBT – 4	37.2	9.9×10^{-2}	0.1	6	1.5		2
n-BA_m-*b*-$GLBT_{n'}$-1	7.08	7.1×10^{-2}	0.57			5	24
n-BA_m-*b*-$GLBT_{n'}$-2	8.5	2.8×10^{-2}	0.27			5	24
n-BA_m-*b*-$GLBT_{n'}$-3	21.24	4.3×10^{-2}	0.34			6	24
n-BA_m-*b*-$GLBT_{n'}$-4	6.23	1.1×10^{-2}	0.39			5	24

[a]For synthesis of diblock copolymers, amount of macro RAFT agent in mmol. [b]for synthesis of homopolymers and diblock copolymers, 4,4′-Azocyanovaleric acid (ACVA) and 2,2′-azobisisobutyronitrile (AIBN) were respectively used as initiator. DMF: dimethylformamide. GLBT: carboxybetaine. *n*-BA: *n*-butylacrylate.

Table 2. Properties of homopolymers (PGLBT) and diblock copolymers (P*n*-BA-*b*-PGLBT)

Polymer	M_n[a] (g/mol)	PDI[b]	Degree of polymerization	
			m	*n'*
PGLBT – 1	11900	1.13		55
PGLBT – 2	25200	1.2		117
PGLBT – 3	33600	1.15		156
PGLBT – 4	64500	1.16		300
n-BA_m-*b*-$GLBT_{n'}$-1			47	55
n-BA_m-*b*-$GLBT_{n'}$-2			88	117
n-BA_m-*b*-$GLBT_{n'}$-3			104	156
n-BA_m-*b*-$GLBT_{n'}$-4			254	300

[a]M_n: Number averaged molecular weight (determined by GPC). [b]PDI: Polydispersity index (M_w/M_n) (determined by GPC). *m*, *n'*: The degree of polymerization of hydrophobic and hydrophilic blocks respectively(*m* was determined by NMR). GLBT: carboxybetaine; *n*-BA: *n*-butylacrylate.

Table 3. CMC of different block copolymers determined using SLS and fluorescence probe measurements.

Diblock copolymer	CMC (mg/ml)							
	SLS				Fluorescence			
	Water	0.1M NaCl	0.5M NaCl	1M NaCl	Water	0.1M NaCl	0.5M NaCl	1M NaCl
n-BA_{47}-*b*-$GLBT_{55}$	-	-	-	-	0.015	-	-	0.01
n-BA_{88}-*b*-$GLBT_{117}$	-	-	-	-	0.02	-	-	0.015
n-BA_{104}-*b*-$GLBT_{156}$	0.06	0.0025	0.003	0.0032	-	-	-	-
n-BA_{254}-*b*-$GLBT_{300}$	-	-	-	-	0.022	-	-	0.009

Table 4. Dissymmetry ratio and polydispersity indices of the micelles

Diblock copolymer	Z_d [a]		PDI [b]	
	Water	1M NaCl	Water	1M NaCl
n-BA_{47}-*b*-$GLBT_{55}$	1.8	1.9	0.13	0.14
n-BA_{88}-*b*-$GLBT_{117}$	4.7	4.9	0.22	0.22
n-BA_{104}-*b*-$GLBT_{156}$	4.5	4	0.28	0.28
n-BA_{254}-*b*-$GLBT_{300}$	2.6	2.9	0.14	0.14

[a]Dissymmetry ratio $Z_d = I_{45}/I_{135}$; I_{45} and I_{135} are scattering intensities at angles 45° and 135° respectively. [b]PDI: Polydispersity indices of the micelles.

Table 5. Zimm plots results

Diblock copolymer	N_{agg} [a]		$A_2 \times 10^5$ ($cm^3 g^{-2} mol$) [b]	
	Water	1M NaCl	Water	1M NaCl
n-BA_{47}-*b*-$GLBT_{55}$	755	69	5.4	0.9
n-BA_{254}-*b*-$GLBT_{300}$	155	35	-4×10^{-2}	-2.4

[a] N_{agg}: Aggregation number. [b]A_2: Second virial coefficient.

Table 6. Parameters related to structure of micelles

Diblock copolymer	R_C (nm) [a]		R_h (nm) [b]		R_g (nm) [c]		Contour length of BA (nm) [d]	β [e]	
	Water	1M NaCl	Water	1M NaCl	Water	1M NaCl		Water	1M NaCl
n-BA_{47}-*b*-$GLBT_{55}$	11.86	5.34	63.7	65.1	81.5	85.6	11.9	0.002	0.001
n-BA_{254}-*b*-$GLBT_{300}$	12.28	7.48	79.1	85.8	122.9	140.2	64.5	0.006	0.002

[a] R_C: Core radius. [b]R_h: Hydrodynamic radius. [c]R_g: Radius of gyration.
[d] Contour length of BA = Degree of polymerization of BA x 0.254 nm. [e] β: Packing parameter.

Figure captions:

Figure 1. ^{1}H NMR spectra of (a) homopolymer PGLBT in D_2O, (b) diblock copolymer P(*n*-BA)-*b*-PGLBT in $CDCl_3$:CD_3OD (1:1).

Figure 2. Variation of surface tension of (a) *n*-BA_{47}-*b*-$GLBT_{55}$ (b) *n*-BA_{88}-*b*-$GLBT_{117}$ and (c) *n*-BA_{254}-*b*-$GLBT_{300}$ as a function of their concentrations in water and various concentrations of sodium chloride solutions.

Figure 3. Foam formation observation for the polymer solutions of (a) *n*-BA_{47}-*b*-$GLBT_{55}$, (b) *n*-BA_{88}-*b*-$GLBT_{117}$, (c) *n*-BA_{104}-*b*-$GLBT_{156}$ and (d) *n*-BA_{254}-*b*-$GLBT_{300}$ of concentration (1 mg/ml) in water (top row) and 1M sodium chloride solutions (bottom row).

Figure 4. Variation of foam height of different block copolymer solutions (concentration = 1 mg/ml) as a function of time. (a) in water and (b) in 1M NaCl.

Figure 5. Critical micelle concentration of *n*-BA_{104}-*b*-$GLBT_{156}$ as a function of sodium chloride solutions.

Figure 6. Fluorescence intensity of NPN as a function of polymer concentrations (a) *n*-BA_{47}-*b*-$GLBT_{55}$, (b) *n*-BA_{88}-*b*-$GLBT_{117}$ and (c) *n*-BA_{254}-*b*-$GLBT_{300}$.

Figure 7. Hydrodynamic radius as a function of polymer concentrations (a) *n*-BA_{104}-*b*-$GLBT_{156}$ and (b) *n*-BA_{254}-*b*-$GLBT_{300}$. Inset shows variation of hydrodynamic radius with different concentrations of sodium chloride solutions.

Figure 8. Typical Zimm plots for polymers in 1M NaCl. (a) *n*-BA_{47}-*b*-$GLBT_{55}$ and (b) *n*-BA_{254}-*b*-$GLBT_{300}$.

Figure 9. Schematic representation of effect of salt on the surface active and micellization behaviours of zwitterionic amphiphilic diblock copolymers.